Remarks

In the instant amendment, Applicants cancel claims 4-20, 24-57, 59-65, 67-69 and 71-76 without abandoning Applicants' right to pursue those claims again in the future, and add a new claim 77, which is dependent on claim 58, to further limit plant to *Arabidopsis*. Applicants amend claims 22 and 23 to clarify the subject matter of those claims. Applicants also amend claims 58, incorporating all limitations of the base claim 6. Applicants also amend claims 66 and 70 to place them in independent form, deleting the recitation to SEQ ID NO:2, and incorporating all limitations of the base claim 4 for claim 66, and the base claim 4 and intervening claim 5 for claim 70. As a result, claims 1-3, 21-23, 58, 66, 70 and 77 are currently pending.

The Examiner rejected claims 3, 5, 15, 19, 23, 27, 54-56, 60, 62, 67-70, 72, 74 and 76 under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. The Examiner contended that the amended and newly submitted claims were drawn to a subsequence of SEQ ID NO:1 from nucleotides 80-1024, but the specification and figures only provided basis for a subsequence of SEQ ID NO:1 from nucleotides 81-1024. In particular, the Examiner contended that Figure 2 demonstrated that the start codon in SEQ ID NO: 1 began at nucleotide 80, but the start codon ATG began at nucleotide 81.

Applicants respectfully submit that the Examiner has mis-read SEQ ID NO:1, and the start codon in SEQ ID NO:1 does begin at nucleotide 80; the A immediately preceding the start codon is nucleotide 79. A copy of Figure 2 showing the numbering of the nucleotides is attached. Therefore, reconsideration and withdrawal of the rejection of claim 3 under the written description requirement are requested.

The Examiner rejected claims 18, 22-23 and 26-27 under 35 U.S.C. §112, second paragraph, as being indefinite. Applicants now have cancelled claims 18, 26

and 27 and amended claims 22 and 23. The amendments include the deletion of "an" in the 1st line and "a" in the 3rd line of claim 22, and "a" in the 3rd line of claim 23, upon which the Examiner based the indefiniteness rejection. Thus, it respectfully is submitted that, in light of the foregoing amendments, the indefiniteness rejection of claims 22 and 23 is overcome. Reconsideration and withdrawal of the rejection respectfully are requested.

The Examiner rejected claims 4-6, 54-57 and 59-76 under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. In particular, the Examiner contended that a mere recitation of 70-90% sequence identity, in the absence of any demonstration that said variant sequences would indeed encode a protein having the claimed function, was insufficient. The Examiner further stated that the disclosure of a single gene sequence encoding a single protein only provided an adequate written description for claims drawn to the genus of nucleotide sequences which encoded a protein having at least 95% identity to the entire protein encoded by the exemplified gene.

Applicants disagree with the Examiner's assertion but have cancelled claims 4-6, 54-57, 59-65, 67-69 and 71-76 for purpose of advancing prosecution of this application. As a result, only claims 66 and 70 remain under the rejection based on the written description requirement with regard to the percent identity. Applicants submit that original language of claims 66 and 70 was drawn to the genus of nucleotide sequences which had at least about 95% identity to the entire SEQ ID NOs:1 and 2, rather than a subsequence thereof, and the amended claims 66 and 70, rewritten in independent form and incorporating all limitations of the base and intervening claims, clearly read as such.

The Examiner also rejected claims 4-6, 54-57 and 59-76 on the basis that subsequences with 70%-100% sequence identity to SEQ ID NO:2 are inadequately described since SEQ ID NO:2 is only 271 base pairs long and does not encode a

functional SPOROCYTELESS protein. Applicants have deleted the recitation of SEQ ID NO:2 in claims 66 and 70 without abandoning Applicants' right to pursue claims directed to such sequences again in the future.

Thus, it respectfully is submitted that, in light of the foregoing arguments and amendments, the written description rejection of claims 66 and 70 is overcome. Reconsideration and withdrawal thereof respectfully are requested.

The Examiner rejected claims 4-6, 54-57 and 59-76 under 35 U.S.C. §112, first paragraph, asserting that the specification, while being enabling for claims limited to a nucleic acid from *Arabidopsis thaliana* which comprised the SPOROCYTELESS gene comprising SEQ ID NO:1 (including the coding region from nucleotides 81-1024) or which encodes SEQ ID NO:4 or a protein with at least 95% identity thereto, does not reasonably provide enablement for claims broadly drawn to any nucleic acid from any source which had at least 70% identity to 40mer subsequences of SEQ ID NOS: 1 or 2, or which was characterized only by having at least 70%-100% identity to SEQ ID NO:2 (which itself is only 271 base pairs long).

As Applicants have noted above, claims 4, 6, 54, 57, 59-65, 67-69 and 71-76 have been cancelled; thus of the claims subject to this rejection, only claims 66 and 70 are still pending. These two claims, as noted above, are drawn to sequences having at least 95% identity to the entire SEQ ID NO:1 and the coding region of SEQ ID NO:1 (which is SEQ ID NO:4), respectively.

Further, Applicants submit that the instant application teaches one skilled in the art how to identify whether or not a candidate nucleic acid is involved in meiocyte formation. Example 1 teaches that male or female sterile phenotypes of a plant can be induced using the Ds insertion technique. One skilled in the art then can isolate the mutated gene by transposon tagging. Example 2 teaches that the homology of the gene can be confirmed by sequencing. Examples 5 and 6 teach that one can confirm if

the gene encodes a nuclear protein by in situ localization of the gene's mRNA. Therefore, one can identify a naturally occurring DNA from a plant having a certain percentage of identity to a certain sequence and participating in meiocyte formation in a plant.

In making his rejection, the Examiner cited a reference by Spielman et al. as a reference to show that there is a difference between *Arabidopsis* and other plants (i.e., *Arabidopsis* endosperms can tolerate a degree of paternal excess), and contends that, because of such a difference, one cannot expect that an *Arabidopsis* gene would carry out the same or similar meiocyte formation function in other plants. Such a conclusion, however, is not warranted. There is no teaching or suggestion in the reference that genes related to meiocyte formation in *Arabidopsis* would function in a different way in other plants.

The Examiner also cited a reference by Meinke et al. as support for his non-enablement argument, asserting that this reference states that "the use of Arabidopsis as a model system is still in its infancy" (referring to the bottom paragraph of column 2 at page 681). Applicants respectfully submit that this reference does not make such a statement and that the Examiner's reliance on this reference is misplaced.

The paragraph to which the Examiner referred first summarizes the effort to build a new generation of databases for *Arabidopsis* through an international level of cooperation, and then provides:

> [w]ith continued progress in genomics, biology, and database management, it nevertheless appears likely that *Arabidopsis* will soon become a model not only for understanding plant structure and function, but also for addressing more universal questions concerning the nature and origin of biological complexity (Emphasis added.)

Applicants read this sentence as the *Arabidopsis* research has advanced so much that one skilled in the art will soon use *Arabidopsis* as a model even to figure out more

universal questions concerning the nature and origin of biological complexity beyond simply understanding plant structure and function. Nothing in the Meinke et al. reference states or indicates that the *Arabidopsis* as a plant model system is in its infancy.

Contrary to the Examiner's assertions, Applicants submit that at the time of filing one skilled in the art could expect that a certain gene involved in the formation of flowers or the specification of floral organ identity in one plant would carry out the same function in most other plants. For example, it was known by 1994 that homologous genes in angiosperms had functional interchangeability:

> All angiosperms shares similar lifestyle, environmental challenges, modes of reproduction, and body plans. The roughly 250,000 species of angiosperms are thought to have evolved from a common ancestor within at least 150 million years. Because of this relatively recent evolution, the average *Arabidopsis* gene can be confidently expected to functionally replace a homology in many other angiosperms and can generally be used as a heterologous hybridization probe to isolate the corresponding gene.

Arabidopsis, Cold Spring Harbor monograph series, monograph 27, editors: Elliot M. Meyerowitz and Chris R. Somerville, Cold Spring Harbor Laboratory Press (1994) page 5, the second paragraph (copy enclosed). Furthermore, this general interchangeability was confirmed in 2001:

> Since both the formation of flowers and the specification of floral organ identity are likely to be governed by genetic programmes that are universal within the angiosperms, studies have concentrated on a few genetically tractable model species.

John L. Bowman, *Arabidopsis: Flower Development and Patterning*, Encyclopedia of Life Sciences, page 1 of the article, the first column, the second paragraph (copy enclosed).

For the foregoing reasons, Applicants submit that newly amended claims 66 and 70 are fully enabled. Reconsideration and withdrawal of the instant enablement rejection of claims 66 and 70 are requested.

Claims 13-15, 17-19, 21-23 and 25-27 remain rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement. Claims 13-15, 17-19 and 25-27 now have been cancelled; claims 21-23 are currently pending. Claims 21-23 are drawn to a plant cell transformed with the claimed nucleic acids.

Example 7 of the instant application shows a successful plant cell transformation, in which the SPL gene is expressed. It teaches that there are already standard methods of plant transformation "(e.g., Bethtold, N., and Pelletier, G., Methods Mol. Biol. 82:259-266 (1998))." Page 42, Example 7 of the instant application.

Although the Examiner cited a reference by Matsuoka et al. to show the level of skill in the art at the time of filing, that reference was published in 1993, and Applicants submit that a reference published in 1993 is too old to accurately reflect the level of skill in 1999, especially in an art making progress as rapidly as biotechnology and in light of the standard methods of plant transformation disclosed in the specification as noted above.

Therefore, Applicants submit that claims 21-23, drawn to a plant cell transformed with the claimed nucleic acids, are fully enabled. Reconsideration and withdrawal of the instant enablement rejection of claims 21-23 are requested.

Under 35 U.S.C. §102(b), the Examiner rejected claims 5, 72, 74 and 76 as being anticipated by Rounsley et al, and claims 4-5, 54-56, 59-65 and 67-69 as being anticipated by Weigel et al or Pnueli et al. As all of the rejected claims have been cancelled, both of these §102(b) rejections have been rendered moot.

In light of the foregoing amendments and remarks, Applicants respectfully submit that this application is in condition for allowance and such action is earnestly solicited.

If any fees are required with respect to this paper, please charge our Deposit Account 02-2135.

Customer Number or Bar Code Label **6449**			
RESPECTFULLY SUBMITTED,			
NAME AND REG. NUMBER	Barbara G. Ernst, Registration Number 30,377		
SIGNATURE	Barbara G. Ernst	DATE	10/26/04
Address	Rothwell, Figg, Ernst & Manbeck 1425 K Street, N.W., Suite 800		
City	Washington	*State*	D.C.
Country	U.S.A.	*Telephone*	202-783-6040

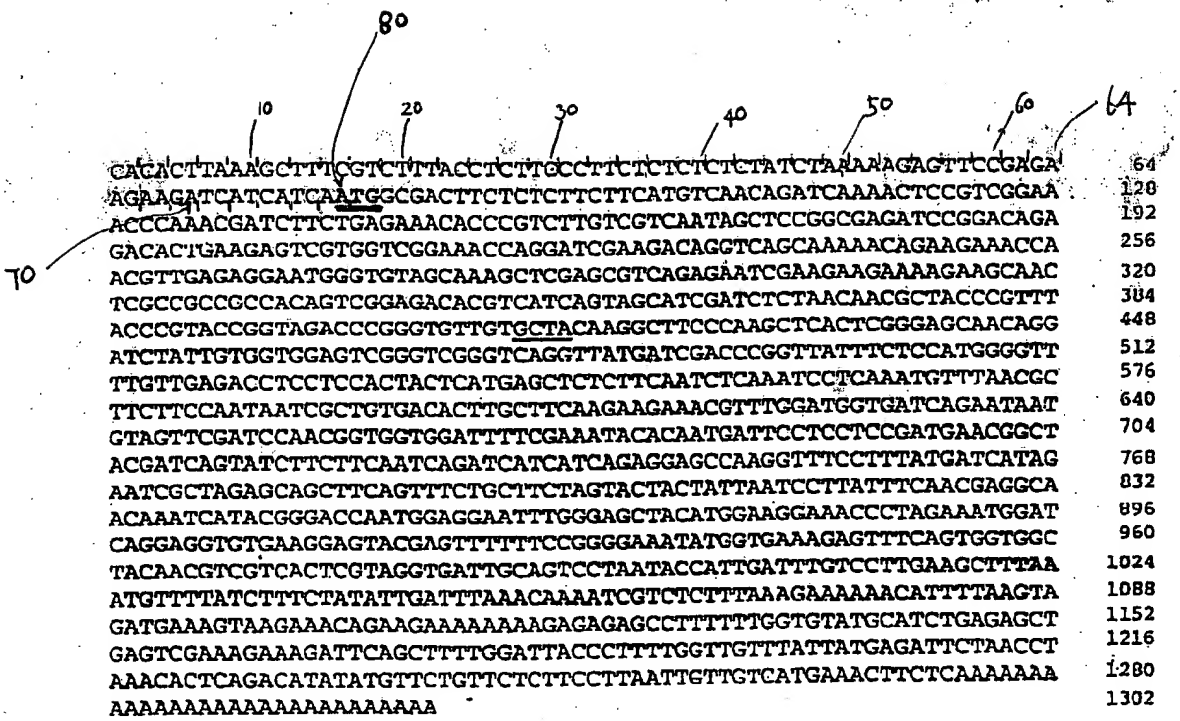
80
10
20
30
40
50
60
64
70
CACACTTAAAGCTTTCGTCTTTACCTCTTGCCTTCTCTCTCTCTATCTAAAAAGAGTTCCGAGA 64
AGAAGATCATCATCAATGGCGACTTCTCTCTTCTTCATGTCAACAGATCAAAACTCCGTCGGAA 128
ACCCAAACGATCTTCTGAGAAACACCCGTCTTGTCGTCAATAGCTCCGGCGAGATCCGGACAGA 192
GACACTGAAGAGTCGTGGTCGGAAACCAGGATCGAAGACAGGTCAGCAAAAACAGAAGAAACCA 256
ACGTTGAGAGGAATGGGTGTAGCAAAGCTCGAGCGTCAGAGAATCGAAGAAGAAAAGAAGCAAC 320
TCGCCGCCGCCACAGTCGGAGACACGTCATCAGTAGCATCGATCTCTAACAACGCTACCCGTTT 384
ACCCGTACCGGTAGACCCGGGTGTTGTGCTACAAGGCTTCCCAAGCTCACTCGGGAGCAACAGG 448
ATCTATTGTGGTGGAGTCGGGTCGGGTCAGGTTATGATCGACCCGGTTATTTCTCCATGGGGTT 512
TTGTTGAGACCTCCTCCACTACTCATGAGCTCTCTTCAATCTCAAATCCTCAAATGTTTAACGC 576
TTCTTCCAATAATCGCTGTGACACTTGCTTCAAGAAGAAACGTTTGGATGGTGATCAGAATAAT 640
GTAGTTCGATCCAACGGTGGTGGATTTTCGAAATACACAATGATTCCTCCTCCGATGAACGGCT 704
ACGATCAGTATCTTCTTCAATCAGATCATCATCAGAGGAGCCAAGGTTTCCTTTATGATCATAG 768
AATCGCTAGAGCAGCTTCAGTTTCTGCTTCTAGTACTACTATTAATCCTTATTTCAACGAGGCA 832
ACAAATCATACGGGACCAATGGAGGAATTTGGGAGCTACATGGAAGGAAACCCTAGAAATGGAT 896
CAGGAGGTGTGAAGGAGTACGAGTTTTTTCCGGGGAAATATGGTGAAAGAGTTTCAGTGGTGGC 960
TACAACGTCGTCACTCGTAGGTGATTGCAGTCCTAATACCATTGATTTGTCCTTGAAGCTTTAA 1024
ATGTTTTATCTTTCTATATTGATTTAAACAAAATCGTCTCTTTAAAGAAAAAACATTTTAAGTA 1088
GATGAAAGTAAGAAACAGAAGAAAAAAAAGAGAGAGCCTTTTTTGGTGTATGCATCTGAGAGCT 1152
GAGTCGAAAGAAAGATTCAGCTTTTGGATTACCCTTTTGGTTGTTTATTATGAGATTCTAACCT 1216
AAACACTCAGACATATATGTTCTGTTCTCTTCCTTAATTGTTGTCATGAAACTTCTCAAAAAAA 1280
AAAAAAAAAAAAAAAAAAAAA 1302

FIG.2

ARABIDOPSIS

Edited by

Elliot M. Meyerowitz
California Institute of Technology, Pasadena

Chris R. Somerville
Carnegie Institution, Stanford, California

COLD SPRING HARBOR LABORATORY PRESS
1994

ARABIDOPSIS

Monograph 27

Printed in the United States of America
Book design by Emily Harste

ISBN 0-87969-428-9
ISSN 0270-1847
LC 94-79111

All Cold Spring Harbor Laboratory Press publications may be ordered directly from Cold Spring Harbor Laboratory Press, 10 Skyline Drive, Plainview, New York 11803. Phone: 1-800-843-4388 in Continental U.S. and Canada. All other locations: (516) 349-1930. FAX: (516) 349-1946.

Contents

Introduction

Chris R. Somerville
Department of Plant Biology
Carnegie Institution
Stanford, California 94305

Elliot M. Meyerowitz
Division of Biology, 156-29
California Institute of Technology
Pasadena, California 91125

The contents and scope of the chapters in this volume attest to the fact that *Arabidopsis thaliana* is a useful and popular model organism for investigating a broad range of topics in plant biology. The rapidity and magnitude of growth in the use of *Arabidopsis* as an experimental organism during the past 10 years is undoubtedly due to a variety of factors. The many advantages of *Arabidopsis* for studies in classical genetics, and in large-scale mutant screens, have long been known and stimulated a burst of enthusiasm for its use as a model for plant genetics in the 1960s. However, perhaps because of the perceived linkage between plant breeding and genetics and the absence of any compelling reasons to work on *Arabidopsis* before the advent of gene cloning, crop species such as maize, tomato, pea, and barley remained the major organisms for genetic studies of plants. A cogent review of the virtues of *Arabidopsis* (Rédei 1975) and the first stirrings of plant molecular biology attracted a small coterie of students in the late 1970s (see, e.g., Meinke and Sussex 1979; Somerville and Ogren 1979; Koornneef et al. 1980). The first genetic map of the organism, which showed the power of large-scale mutagenesis and the ease of performing classical genetic manipulations, was published in 1983 (Koornneef et al. 1983).

The advantages of the organism for studies in molecular biology, which became apparent after the small size and simple structure of the nuclear genome was determined (Leutwiler et al. 1984; Pruitt and Meyerowitz 1986), were spelled out in several reviews (Meyerowitz and Pruitt 1985; Meyerowitz 1987). These papers, and the early and enthusiastic reports of applications of modern methods to *Arabidopsis* (see, e.g., North 1985), attracted large numbers of plant biologists who had only recently begun to incorporate the techniques of molecular

Arabidopsis

biology into their research programs. They also attracted biologists who had been trained in yeast, *Drosophila*, bacteria, or vertebrates but were interested in moving to plants. Reports describing methods for *Arabidopsis* transformation (An et al. 1986; Lloyd et al. 1986; Feldmann and Marks 1987), and examples of the uses of mutants for physiological, biochemical, and developmental studies (Estelle and Somerville 1986), stimulated and consolidated interest at that time.

A second wave of interest in *Arabidopsis* developed several years later as the new discipline of "plant molecular biology" began to mature. As plant biologists became increasingly familiar with genes, a widespread appreciation for the methodological approaches and power of genetics developed in parallel. Although there were large numbers of interesting mutations and a rich heritage of classical genetics in plants such as maize, pea, *Antirrhinum*, and tomato, the technical advantages of *Arabidopsis* for many genetic methods were persuasive. In addition, largely due to the work of Maarten Koornneef and his colleagues at Wageningen, a collection of important mutants affecting floral morphology, phytochrome, phytohormone responses, and flowering time was freely available. These and other mutants from the collections of Albert Kranz and George Rédei, among others, served as a kind of "starter kit" that allowed many laboratories to begin genetic investigations of problems of broad importance without the delays associated with isolating and characterizing new mutants. Perhaps the clinching attraction was the development of a method for producing large numbers of T-DNA insertions (Feldmann and Marks 1987) and the dissemination of a large collection of T-DNA insertion mutants by Ken Feldmann and colleagues. The ease with which genes could be cloned from this collection proved irresistible to the large number of people fortunate enough to find their favorite mutation in the collection.

Whatever the precise reasons for the large number of converts, one effect of having large numbers of scientists using *Arabidopsis* was that it became worthwhile to develop organism-specific tools and infrastructure. The development of molecular marker maps, recombinant inbred lines, and YAC libraries could be justified by the large number of potential users. Once such resources were available, they became additional reasons to use *Arabidopsis* as an experimental organism. It was fortunate for plant biology that a number of granting agency administrators around the world also realized the potential of *Arabidopsis* at an early stage and provided both financial and administrative support not only for individual research projects, but also for workshops and community-wide resources such as stock centers, databases, and the EST projects. Some of the U.S. administrators who deserve special mention in this respect are

Machi Dilworth, Delill Nasser, and Mary Clutter of the National Science Foundation and Bob Rabson and Greg Dilworth of the Department of Energy. Étienne Magnien of the Commission of the European Communities, Directorate of Biology, played a similarly influential role in Europe.

As a result, we are now in a "golden age" of discovery in plant biology. Problems that have been intractable for decades are yielding to the application of modern methods in molecular and cellular biology. The formula for much of this success is conceptually simple: Isolate a mutation that affects the process or structure of interest, clone the gene, find out where and when it is expressed, where the gene product is located, what it does, and what it interacts with, directly or indirectly. The adaptation of this "molecular genetic paradigm" to problems in plant biology is not species-specific but has been greatly facilitated by the widespread adoption of *Arabidopsis* as a model species. Large numbers of interesting and informative mutations are now available, and the list of mutations is not only increasing rapidly but is increasing in the sophistication of the criteria used to identify the mutations. Now that the promise of map-based cloning has been realized by a number of laboratories, it seems that everything is within grasp. Although it is not necessarily easy, any gene that can be marked by a mutation can be cloned. This is a qualitatively different situation from anything that has ever before existed in plant biology and is, for the moment, unique to *Arabidopsis*.

Having come this far, it seems an opportune time to pose the question of where we are bound. Because the *Arabidopsis* community is now organized on an international scale with elected representatives and an electronic forum for rapid dissemination of information and discussion of issues (Dennis et al. 1993), it is possible to undertake large-scale projects that serve the community as a whole or that require broad participation. In this respect, the goal of sequencing the entire genome is a logical next step. Indeed, the first tentative steps toward large-scale genomic sequencing have been taken in Europe, and representatives of the U.S. *Arabidopsis* community have endorsed the initiation of a complementary U.S. project. Thus, we may already imagine that by 2004 it will be possible to sit down at a computer and call up the entire sequence of a plant genome. It is not too early to begin thinking about the consequences and possibilities inherent in this eventuality.

An attractive model for large-scale sequencing is the *Caenorhabditis elegans* sequencing project. The members of this project release the data to the community as it is produced (as opposed to the yeast sequencing projects, which release whole chromosomes). Thus, the community can exploit the information as it is produced. Although it will be several

years before the large-scale *Arabidopsis* sequencing is producing comparable levels of useful information, in the meantime, it seems likely that sequence information will be available for the vast majority of expressed genes via the EST sequencing projects (Höfte et al. 1993; Newman et al. 1994). These projects have already produced more than 8000 partial cDNA clones that have, in turn, permitted the identification of more than 1000 genes of identifiable function by reference to the sequences in the public databases. It also seems likely that most of the ESTs will be placed on the genetic map and that a set of overlapping YAC clones will soon be available.

Having the sequence of the genome will not identify the function of all the genes, but it will certainly provide new tools for analyzing gene function. Presumably, we will find ourselves in a situation analogous to the current situation with the *Saccharomyces* genome project. Sequencing of the first few yeast chromosomes revealed that mutational analysis of yeast had identified only about 20% of the genes. Thus, much of the mutational analysis of yeast will now proceed by directed mutagenesis. The availability of the total genomic sequence is essential to this because it permits identification of other copies of structurally related genes in the genome that may also need to be inactivated in order to assess the gene function. Similarly, we may expect that directed gene inactivation of *Arabidopsis* will become an increasingly important aspect of research on *Arabidopsis*. One efficient way of accomplishing this may be the development of a comprehensive set of insertional mutants that can be screened by PCR methods to identify the desired insertion. Under optimal conditions, this approach could permit one to have seed of a desired insertional mutant in hand within a few days from the start of an experiment. There is no theoretical reason why directed gene knockouts cannot be done as well, although methods for doing these efficiently have yet to be found.

In parallel with the elucidation of genome structure, we envision the development of new generations of computer resources. The role of relatively simple databases in managing sequence and mapping information can be seen from contemporary models. However, there is no apparent reason why all other information about *Arabidopsis* cannot also be organized in a database. Imagine the advantages of having all the information *summarized* in this volume available in a relational database! In principle, the computing tools already exist so that all overlapping information can be linked.

As our knowledge of plant biology grows and is refined, it will increasingly be necessary to connect the artificially fragmented aspects of biological knowledge into a seamless and interactive whole that explains

the overall properties of the organism. Our goal should be to have such a deep understanding of the biology that we can confidently predict, from first principles, how a change in one environmental parameter, or the activity of a particular gene, will affect the growth and development of the organism. Such methods could also be applied to learning how a change in a gene or in its activity might have had (or might yet have) a particular impact during the evolution of the organism. It is to be hoped that, as one of the forefront biological systems, *Arabidopsis* will continue to attract researchers with a primary interest in bioinformatics and knowledge systems. Eventually, it should be possible to sit down in front of a computer and ask complex "What if" questions and get back synthetic responses in which the computer synthesizes specific answers from predictive models of all aspects of the biology of the organism. The inability of a computer to answer such a query might become a criterion for pursuing an experiment.

The purpose of an organism as an experimental "model system" is to efficiently understand the organisms being modeled. *Arabidopsis* differs from several of the other important model systems in at least one key respect. Unlike yeast, which is taken as a model for all eukaryotes, or *C. elegans* or *Drosophila*, which are considered to be useful models for animal development, *Arabidopsis* is closely related to the species it models. All angiosperms share similar life-styles, environmental challenges, modes of reproduction, and body plans. The roughly 250,000 species of angiosperms are thought to have evolved from a common ancestor within the last 150 million years. Because of this relatively recent evolution, the average *Arabidopsis* gene can be confidently expected to functionally replace a homolog in many other angiosperms and can generally be used as a heterologous hybridization probe to isolate the corresponding gene. One implication of this high degree of similarity between the model and the modeled is that all aspects of *Arabidopsis* biology—developmental, metabolic, biochemical, environmental, and so on—are worthy of investigation because of the broad applicability of the information. A second implication is that there are many opportunities to exploit detailed knowledge about *Arabidopsis* to gain insights into similar processes in other plants. Thus, biologists with a primary interest in another plant species will find it increasingly useful to have a deep understanding of the resources and knowledge produced through research on *Arabidopsis*. This volume is a step in attempting to make this knowledge accessible to all plant biologists and, in fact, to make it available to all biologists interested in eukaryotic organisms, in the interactions of eukaryotes with prokaryotes, and in the profound differences between plants and animals.

REFERENCES

An, G., B.D. Watson, and C.C. Chiang. 1986. Transformation of tobacco, tomato, potato, and *Arabidopsis thaliana* using a binary Ti vector system. *Plant Physiol.* **81:** 301–305.

Dennis, L., C. Dean, R. Flavell, H. Goodman, M. Koornneef, E. Meyerowitz, Y. Shimura, M. van Montagu, and C. Somerville. 1993. The multinational coordinated *Arabidopsis thaliana* genome research project progress report: Year three. *U.S. National Science Foundation Publ.* NSF 93-173.

Estelle, M.A. and C.R. Somerville. 1986. The mutants of *Arabidopsis*. *Trends Genet.* **2:** 89–93.

Feldmann, K.A. and M.D. Marks. 1987. *Agrobacterium*-mediated transformation of germinating seeds of *Arabidopsis thaliana*: A non-tissue culture approach. *Mol. Gen. Genet.* **208:** 1–9.

Höfte, H., T. Desprez, J. Amselem, H. Chiapello, M. Caboche, A. Moisan, M.F. Jourjon, J.L. Charpenteau, P. Berthomieu, D. Guerrier, J. Giraudat, F. Quigley, F. Thomas, D.Y. Yu, R. Mache, M. Raynal, R. Cooke, F. Grellet, M. Delseny, Y. Parmentier, G. Marcillac, C. Gigot, J. Fleck, G. Philipps, M. Axelos, C. Bardet, D. Tremousaygue, and B. Lescure. 1993. An inventory of 1152 expressed sequence tags obtained by partial sequencing of cDNAs from *Arabidopsis thaliana*. *Plant J.* **4:** 1051–1061.

Koornneef, M., E. Rolff, and C.J.P. Spruit. 1980. Genetic control of light-induced hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh. *Z. Pflanzenphysiol.* **106:** 147–160.

Koornneef, M., J. Van Eden, C.J. Hanhart, P. Stam, F.J. Braaksma, and W.J. Feenstra. 1983. Linkage map of *Arabidopsis thaliana*. *J. Hered.* **74:** 265–272.

Leutwiler, L.S., B.R. Hough-Evans, and E.M. Meyerowitz. 1984. The DNA of *Arabidopsis thaliana*. *Mol. Gen. Genet.* **194:** 15–23.

Lloyd, A.M., A.R. Barnason, S.G. Rogers, M.C. Byrne, R.T. Fraley, and R.B. Horsch. 1986. Transformation of *Arabidopsis thaliana* with *Agrobacterium tumefaciens*. *Science* **234:** 464–466.

Meinke, D.W. and I.M. Sussex. 1979. Embryo-lethal mutants of *Arabidopsis thaliana*: A model system for genetic analysis of plant embryo development. *Dev. Biol.* **72:** 50–61.

Meyerowitz, E.M. 1987. *Arabidopsis thaliana*. *Annu. Rev. Genet.* **21:** 93–112.

Meyerowitz, E.M. and R.E. Pruitt. 1985. *Arabidopsis thaliana* and plant molecular genetics. *Science* **229:** 1214–1218. (Reprinted [1986] in *Biotechnology: The renewable frontier* [ed. D.E. Koshland, Jr.], pp. 311–320. AAAS, Washington, D.C.)

Newman, T., F.J. de Bruijn, P. Green, K. Keegstra, H. Kende, L. McIntosh, J. Ohlrogge, N. Raikhel, S. Somerville, M. Thomashow, E. Retzel, and C.R. Somerville. 1994. Genes galore: A summary of methods for accessing results from large-scale partial sequencing of anonymous *Arabidopsis* cDNA clones. *Plant Physiol.* (in press).

North, G. 1985. A plant joins the pantheon at last? *Nature* **315:** 366–367.

Pruitt, R.E. and E.M. Meyerowitz. 1986. Characterization of the genome of *Arabidopsis thaliana*. *J. Mol. Biol.* **187:** 169–183.

Rédei, G.P. 1975. *Arabidopsis* as a genetic tool. *Annu. Rev. Genet.* **9:** 111–127.

Somerville, C.R. and W.L. Ogren. 1979. A phosphoglycolate phosphatase-deficient mutant of *Arabidopsis*. *Nature* **280:** 833–836.

Arabidopsis: Flower Development and Patterning

John L Bowman, *University of California, Davis, California, USA*

Introductory article

Article Contents

The development of flowers and floral organs is directed by genetic programmes likely to be universal amongst the angiosperms. A simple model explaining how a small number of regulatory genes acting alone and in combination to specify the identity of the floral organs (sepals, petals, stamens and carpels) is supported by both molecular and genetic experiments in *Arabidopsis*.

Introduction

Flowers are one of the defining features of the angiosperms, the dominant group of land plants today. A universal theme underlies the enormous diversity in the size, shape and complexity amongst the flowers of the quarter of a million extant species of angiosperms. Flowers are built of four basic organ types (sepals, petals, stamens and carpels) arranged in concentric rings, called whorls. Sepals occupy the outermost whorl, with petals, stamens and carpels occupying successively more interior positions. Thus, a basic ground plan exists for organ type and position in all angiosperm flowers. Because of the constancy in the relative positions of floral organ types, it is hypothesized that a common genetic programme to specify floral organ identity is utilized during the development of all flowers.

Mutations affecting flowers and their organs provide a powerful means for studying the genetic interactions involved in their development. Differences in the development of mutant versus normal (wild-type) plants reveal the function of the mutated gene. Subsequent cloning of such genes then reveals the nature of their biochemical function. Since both the formation of flowers and the specification of floral organ identity are likely to be governed by genetic programmes that are universal within the angiosperms, studies have concentrated on a few genetically tractable model species. One such species, *Arabidopsis thaliana*, has a number of attributes that facilitate molecular genetic experiments. These attributes include a short generation time (about 6–8 weeks), ease of self- and cross-fertilization, ease of mutant isolation, ease of transformation to generate transgenic plants, and a small genome.

Establishment of the Floral Meristem

Arabidopsis is an annual plant. After seed germination, a small number of leaves are produced from a meristem at the tip of the shoot, referred to as the shoot apical meristem. This is the plant's vegetative stage of growth. Leaves exhibit a 'spiral phyllotaxy', arising one after another from the flanks of the meristem in a spiral pattern. After producing a rosette of leaves, the plant switches from vegetative to reproductive growth so that the apical meristem becomes an inflorescence meristem and starts producing flower meristems. This switch from vegetative to reproductive growth is sensitive to both environmental and endogenous signals and is timed to maximize seed set in the environment in which *Arabidopsis* evolved. Flower meristems are initiated in an indeterminate spiral from the flanks of the inflorescence meristem (**Figure 1a**). Flower meristems are determinate, producing a single flower each. Since the developmental fates of the flower and inflorescence meristems differ, there must exist gene products that distinguish newly formed flower meristem cells from cells of the inflorescence meristem from which they were recently derived. In *Arabidopsis* three genes, *LEAFY* (*LFY*), *APETALA1* (*AP1*), and *CAULIFLOWER* (*CAL*) function to specify flower meristem identity. Mutations in each of these genes lead to a partial loss of flower meristem identity, with the meristems produced from the flanks of mutant inflorescence meristems having both flower and inflorescence meristem characteristics.

Mutations in *AP1* result in a partial loss of flower meristem identity. In *ap1* mutants, the positions normally occupied by single flowers are instead occupied by branched structures composed of multiple flowers. (Capitalized italics (*AP1*) denotes the wild-type gene, whereas small italics (*ap1*) denotes the mutant version of the gene.) This phenotype has been interpreted as a partial loss of flower meristem identity. Mutations at a second locus,

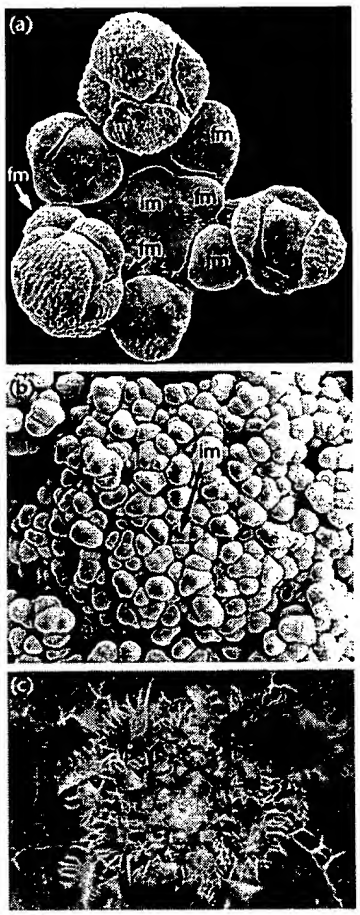

Figure 1 The establishment of the floral meristem. (a) Wild-type inflorescence meristem (im) and young flower meristems (fm). (b) *ap1 cal* inflorescence apex. The apical inflorescence meristem (im) is indicated. (c) *ap1 lfy* inflorescence apex.

CAL, dramatically enhance the phenotypes of *ap1* mutations, making the interpretation of the mutant phenotype unambiguous. In *ap1 cal* double mutant plants, those cells that would normally comprise a flower meristem instead behave as if they constituted an inflorescence meristem. In turn these meristems produce further meristems in a phyllotactic spiral that also behave as inflorescence meristems. This pattern of development can be reiterated several times, resulting in structures composed of large numbers of inflorescence meristems (**Figure 1b**). This phenotype is strikingly similar to that of *Brassica oleracea* var. *botrytis*, the cultivated cauliflower. Indeed, it has been shown that the cultivated cauliflower has a mutation in its *CAL* gene, indicating that the similarity in phenotype is due to similar changes in the genes of the two species. In *Arabidopsis*, *cal* mutations alone have no visible phenotypic effect, suggesting that the functions of *CAL* are fully redundant with those of *AP1*. The functional redundancy of these two genes can be explained at the molecular level. Both *AP1* and *CAL* encode closely related members of a gene family called the MADS box family. MADS box genes encode transcription factors, proteins that regulate the expression of other genes, and members of the family are characterized by a protein motif termed the MADS domain that binds DNA. Thus, both *AP1* and *CAL* code for proteins with very similar biochemical activities. Furthermore, the expression patterns of the two genes are essentially indistinguishable. Each of the genes is expressed at the messenger RNA (mRNA) level throughout incipient flower meristems, but not inflorescence meristems or leaves (**Figure 2b**). In conclusion, the expression pattern and identity of the proteins encoded by the *AP1* and *CAL* genes are consistent with the hypothesis that the function of these genes is to specify flower meristem identity. However, even in *ap1 cal* double mutants, flowers are eventually formed after many reiterations of inflorescence meristem production. This indicates that there must exist other genes that are capable of specifying flower meristem identity in *Arabidopsis*.

Mutations in the *LFY* gene also result in a partial conversion of flower meristems into inflorescence meristems; however, the phenotypes of the partial conversions differ dramatically from those seen in *ap1* mutants. First, bract-like organs subtend each of the partially converted meristems in *lfy* mutants. Bracts are organs that subtend flowers in many families of flowering plants, but are absent from those of the Brassicaceae. Thus, one function of the wild-type *LFY* gene is to suppress bract formation. Second, the flowers that eventually form in *ap1* and *ap1 cal* mutants have normal whorled phyllotaxy (as exhibited by wild-type flower meristems), stamens and carpels, but altered sepals and petals (see below). In contrast, the flowers that develop in *lfy* mutants lack petals and stamens and exhibit a spiral phyllotaxy (as exhibited by wild-type inflorescence meristems). The *lfy* mutant phenotype has also been interpreted as a partial loss of flower meristem specification, with a partial conversion of flowers into inflorescence shoots and an additional failure to suppress bract formation. Again, multiple mutant analyses provides a more lucid interpretation of the phenotypes. In *ap1 lfy* double mutants or *ap1 lfy cal* triple mutants the cells that would normally be destined to become flower meristems, instead behave as if they constituted inflorescence meristems (**Figure 1c**). In addition, these inflorescence meristems are subtended by leafy bract-like organs. Each of these inflorescence meristems then reiterates the developmental pattern described above, such that the plants produce indeterminate leafy shoots composed of inflorescence meristems, rather than flowers. Thus, the partial transformations of flower meristems into inflorescence meristems seen in the *ap1* and *lfy* single mutants become complete transformations in the *ap1 lfy* and *ap1 lfy cal* multiple mutants, suggesting that these genes work cooperatively and in a partially redundant manner to specify flower meristem identity. The *LFY* gene also

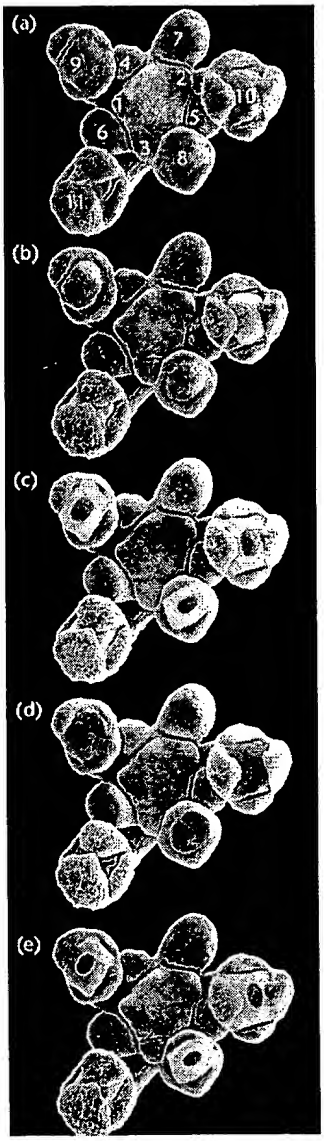

Figure 2 Expression patterns of flower meristem identity and floral homeotic genes. (a) *LFY* (purple) is expressed in floral meristem anlagen (1, 2), flower meristems (3–7), and young developing flowers (8–11). (b) The A class gene *AP1* (red) is expressed in flower meristems, developing sepals and petals in whorls one and two of the flower, and the floral pedicel. (c) The B class genes *AP3* and *PI* (yellow) are expressed in whorls 2 and 3 which will develop into petals and stamens. (d) The C class gene *AG* (blue) is expressed in whorls three and four which will develop into stamens and carpels. (e) Composite of B, C, and D; in whorl one A class genes are expressed alone (red), in whorl two both A and B class genes are expressed (orange), in whorl three both B and C class genes are expressed (green), and in whorl four, C class genes are expressed alone (blue).

encodes a transcription factor, but it is not a member of the MADS box family. Consistent with its proposed role, the *LFY* gene is also expressed in incipient flower meristems, but not in inflorescence meristems (**Figure 2a**). The initial timing of *LFY* expression precedes that of *API* and *CAL* and there is genetic evidence that *LFY* is in part responsible for the activation of *API* and *CAL*. Thus, a genetic hierarchy exists in the specification of flower meristem identity; *LFY* is at the apex of the hierarchy with *API* and *CAL* acting subsequently. The action of all genes is required for the proper specification of flower meristem identity.

Another approach to understanding gene function is to express cloned genes ectopically, i.e. at times and/or in places where the genes are not normally expressed. This can be done by putting a gene under control of a constitutively active promoter. When either *API* or *LFY* is expressed constitutively (in all tissues) meristems that would normally behave as inflorescence meristems now act as flower meristems. This phenotype is the opposite of that observed when the genes are in mutant form and corroborates the hypothesis that these genes specify flower meristem identity. Two other features of the transgenic plants constitutively expressing either *API* or *LFY* are noteworthy. First, the transgenic plants still produce a small number of leaves before the apical meristem develops into a flower meristem. This suggests that during the vegetative phase of growth the shoot apical meristem is not immediately competent to respond to expression of the flower meristem identity genes. Second, the transgenic plants make the transition from vegetative to reproductive development earlier than do wild-type plants. A shortened vegetative phase has also been demonstrated when *LFY* is constitutively expressed in other distantly related angiosperms, such as aspen and tobacco. Such reductions in flowering time are of great interest to plant breeders working with species in which flowering occurs only after several years. In the case of aspen trees the reduction in flowering time, from approximately a decade to six months, is particularly striking.

Floral Homeotic Genes

Once a group of cells produced on the flanks of the inflorescence meristem is specified to become a flower by the action of the flower meristem identity genes, the flower meristems initiate the production of floral organs. The *Arabidopsis* flower consists of four concentric whorls of organs (**Figure 3a,b**). Four sepals occupy positions in the first whorl, with the second whorl consisting of four petals. Six stamens containing pollen sacs occupy the third whorl, and a two-carpelled gynoecium containing ovules forms the fourth whorl. The application of molecular genetics has been particularly useful in elucidating how the four types of floral organs acquire their identity. Studies have focused on a particular set of homeotic mutations. In homeotic mutants normal organs develop in the positions where organs of another type are typically found. Specifically, the *Arabidopsis* floral homeotic mutations result in transfor-

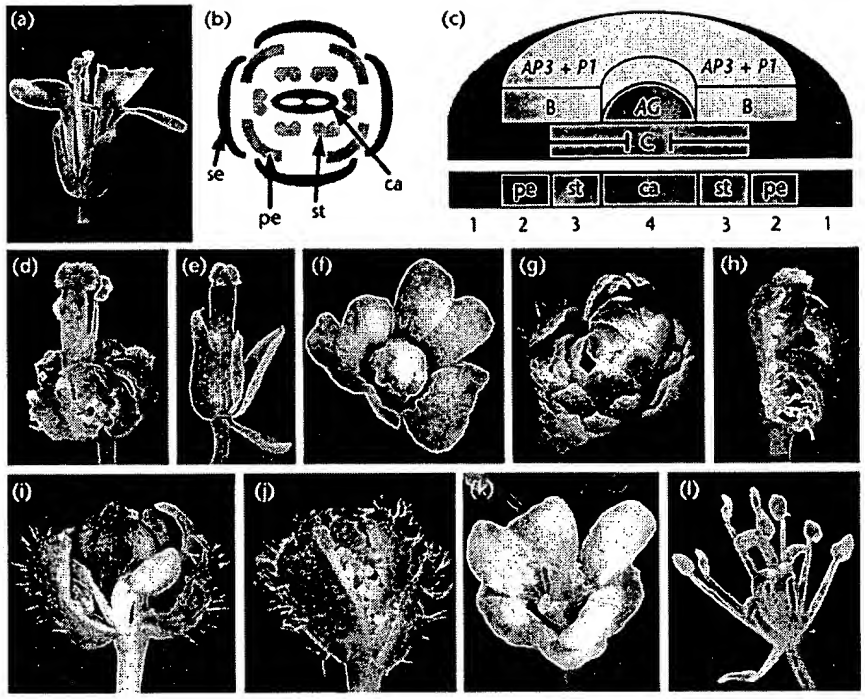

Figure 3 The specification of floral organ identity. (a) Wild-type flower (se, pe, st, ca). (b) Floral diagram of the wild-type flower. (c) The ABC model of the specification of floral organ identity depicting how three classes of floral homeotic genes could specify the identity of each of the four whorls of floral organs. A section through a floral primordium is represented as a set of boxes, with the regions representing each whorl shown at the bottom of each column. In the top set of boxes, each box represents a single field (A (red), B (yellow), or C (blue)). The distribution of floral homeotic gene products present in each of the whorls is shown in the boxes, with the phenotype of the organs in each whorl shown under lower set of boxes: sepal (red), petal (orange), stamen (green), carpel (blue). (d) *ap2* flower (ca, st, st, ca). (e) *pi* flower (se, se, ca, ca). (f) *ag* flower ((se, pe, pe)$_n$). (g) *pi ag* flower ((se, se, se)$_n$). (h) *ap2 pi* flower (ca, ca, ca, ca). (i) *ap2 ag* flower ((le-ca, pe-st, pe-st)$_n$). (j) *ap2 pi ag* flower ((le-ca, le-ca, le-ca)$_n$). (k) Wild-type flower in which the B class genes are constitutively expressed (pe, pe, st, st). (l) *ap2* flower in which the B class genes are constitutively expressed (st, st, st, st). se, sepal; pe, petal, st, stamen, ca, carpel; le-ca, leaf-like carpel; pe-st, petaloid stamen.

mations of one floral organ type into another floral organ type. For example, in one class of mutants, sepals develop in the second whorl positions which are normally occupied by petals, and carpels develop in the third whorl positions which are normally occupied by stamens. One interpretation of these mutants is that cells in the developing flower misinterpret their position, and thus differentiate into inappropriate cell types. In the specific case mentioned above, it is presumed that the function of the wild-type gene product is to specify the identities of the second and third whorl organs as petals and stamens, respectively. In the absence of this gene product, the cells in the second and third whorls misinterpret their positions and differentiate as if they were in the first and fourth whorls, into sepals and carpels, respectively.

The floral homeotic mutants of *Arabidopsis* fall into three classes, designated A, B and C, and mutants in each class result in organ identity defects in two adjacent whorls. Mutations in A class genes (*APETALA2* (*AP2*) and *APETALA1* (*AP1*)) have homeotic conversions in the outer two whorls. In the case of *ap2*, the first whorl organs develop as carpels rather than sepals, and the second whorl organs are either absent or develop as stamens rather than petals (**Figure 3d**). In contrast, in *ap1* mutants the first whorl organs are bract-like, and the second whorls organs are most frequently absent. Note that *AP1* is involved in both the specification of flower meristem identity and in the specification of floral organ identity. That the same gene is used multiple times in development is a common theme in biological systems; it appears to be easier to modify an existing system than to generate a new system *de novo* in evolution. Mutations in either of the two B class genes (*PISTILLATA* (*PI*) and *APETALA3* (*AP3*)) lead to alterations in the middle two whorls, with sepals developing instead of petals in second whorl positions and carpels instead of stamens in third whorl positions (**Figure 3e**). There is only a single identified C class gene (*AGAMOUS* (*AG*)). The inner two whorls are affected in C class mutants, with petals developing in place of stamens in the third whorl while the cells normally fated to become the fourth whorl carpels instead behave as if they constituted another flower meristem (**Figure 3f**). This new flower meristem reiterates the developmental process such that *ag* flowers are a series of nested flowers within flowers.

Thus, the *ag* mutant phenotype can be summarized as (sepal, petal, petal)$_n$ with respect to organ identity. Mutants with similar phenotypes, that of indeterminate whorls of petals, are seen in many horticultural plants, such as roses and carnations, and these may be the result of alterations in C class function itself, or its regulation.

The ABC model

The study of double mutants revealed that B class activity is independent of both A class and C class activities, but that the A class and C class activities are mutually antagonistic. Combining these observations with the activity domains deduced from the single mutant phenotypes led to the formulation of the ABC model of the specification of floral organ identity (**Figure 3c**). The basic tenets of the ABC model are as follows: (1) each of the classes of homeotic gene function acts in a field comprised of two adjacent whorls: A class activity in whorls 1 and 2, B class activity in whorls 2 and 3, and C class activity in whorls 3 and 4; (2) the combination of floral homeotic gene activities present in any particular whorl specifies the type of organ that develops in that whorl, e.g. A class alone specifies sepals, classes A + B specify petals, classes B + C specify stamens, and C class alone specifies carpels; and (3) the class A and class C activities are mutually antagonistic such that loss of A results in C activity in all four whorls and vice versa.

The third postulate is based on the novel phenotype observed in the *ap2 ag* double mutant in which both A and C class activities have been compromised. *ag* mutations alter the phenotype of first and second whorl organs in an *ap2* mutant background, while they do not have a phenotypic effect in these whorls in an otherwise wild-type background. Conversely, while *ap2* mutations do not have a phenotypic effect in third and fourth whorls in an otherwise wild-type background, they alter the phenotype of third and fourth whorl organs in an *ag* mutant background. These observations suggest that C class activity, which is normally only present in whorls three and four, is also present in the outer two whorls in an *ap2* mutant background. Likewise, A class activity is present in the inner two whorls of *ag* mutants. Thus, the A and C class activities are mutually antagonistic, such that removal of one of the activities allows the other to occupy all four whorls and vice versa.

The model successfully predicts the phenotypes of the floral organs for the other double mutant combinations. In *pi ag* (or *ap3 ag*) double mutants, in which both B and C class activities have been lost, only A class activity remains, and A class activity is found in all floral whorls due to the absence of the antagonistic C class activity. Since A class alone specifies sepals, the prediction is that *pi ag* flowers should consist entirely of sepals, and indeed, this is the phenotype observed (**Figure 3g**). Conversely, *ap2 pi* double mutant flowers lack both A and B class activities, leaving only C class activity in all floral whorls. Thus, *ap2 pi* flowers consist entirely of carpels (**Figure 3h**).

In the possible multiple mutant combinations there are two situations in which the identity of the organs is not directly predicted by the model. One case occurs in *ap2 ag* double mutants in which both the A and C class activities are missing, leaving only B class activity in the second and third whorls. B class activity does not normally occur alone, but rather acts in combination with A and C class activities to specify petal and stamen identity, respectively. The second and third whorl organs of *ap2 ag* flowers are neither wild-type petals nor wild-type stamens, but instead have characteristics of both petals and stamens at both the organ and individual cell levels (**Figure 3i**). Thus, these organs, petal–stamen blends, represent a type that is not normally found in wild-type flowers. Likewise, in the first and fourth whorls of *ap2 ag* double mutants and in all whorls of *ap2 pi ag* triple mutants (**Figure 3j**), where none of the A, B or C classes of floral homeotic activities are present, the model does not predict which type of organ will develop. One might suppose that if all genes required for the specification of floral organ identity were removed, the resulting organs might represent a ground state, perhaps a leaf-like organ. However, in these positions organs with features of both carpels and leaves develop. Thus, the organs are somewhat leaf-like, but their carpelloid properties also indicate that there exist additional genes that specify carpel development in the absence of C class gene function.

The floral homeotic genes encode transcription factors

All of the known floral homeotic genes encoding the A, B and C functions have been cloned in *Arabidopsis*, and encode transcription factors. Intriguingly, one of the A class genes (*API*) and all of the B and C class genes (*AP3*, *PI*, *AG*) encode transcription factors belonging to the MADS box gene family, suggesting that a diversification of this gene family may have been instrumental in the evolution of flowers. *AP2* also encodes a transcription factor but of a different family.

The cloning of the floral homeotic genes has allowed molecular tests of the ABC model. For most of the genes it has been shown that the first and third tenets of the model are satisfied at the transcriptional level: each of the genes is expressed in the whorls affected in the corresponding mutant (**Figure 2**). While the expression of *API* is initially throughout the flower meristem, by the time the sepal primordia are forming its expression becomes restricted to the first and second whorls (**Figure 2b**). Simultaneous with the restriction of *API* expression is the initiation of *AG* expression in whorls three and four (**Figure 2d**). It is likely that *AG* directly negatively regulates *API*; *API* may be one

of the targets of the transcriptional control exerted by the *AG* gene product. While *AG* expression is confined to the third and fourth whorls in wild-type flowers, in *ap2* mutant flowers, *AG* is expressed in all floral whorls. These observations begin to shed light on the nature of the mutual antagonism of the A and C class activities. The C class gene *AG* negatively regulates the transcription of the A class gene *AP1* in the third and fourth whorls, and the A class gene *AP2* negatively regulates the transcription of the C class gene *AG* in the first and second whorls. However, not all of the mutual antagonism is at the transcriptional level since *AP2* is transcribed in all four whorls indicating that the restriction of its organ identity activity to the outer two whorls must occur posttranscriptionally. The expression of the class B genes, *AP3* and *PI*, is restricted to the second and third whorls, and their expression is independent of the A and C class genes (**Figure 2c**).

Manipulation of floral organ identity in *Arabidopsis*

Ectopic expression studies of the B and C class genes have shown that they are sufficient to specify organ identity within the flower. For example, ectopic expression of both B class genes in an otherwise wild-type background (**Figure 3k**) results in a flower in which the first and second whorls are occupied by petals (A + B) and the third and fourth whorls are occupied by stamens (B + C). By utilizing combinations of mutant and transgenic lines in which the A, B or C genes have been ectopically expressed it is possible to manipulate organ identity in each whorl of the flower. In practice, it seems that there are few constraints on the identities and positions of the different floral organs. Flowers consisting entirely of sepals or carpels are formed in the *ag ap3* (**Figure 3g**) and *ap2 pi* (**Figure 3h**) double mutants, respectively. Flowers consisting of all petals or all stamens can also be generated. Expression of the B class genes in all four whorls of a C class mutant (*ag*) results in a flower consisting of petals in all whorls. Conversely, expression of the B class genes in all four whorls of an A class mutant (*ap2*) results in a flower consisting of stamens in every whorl (**Figure 3i**). Thus, any of the different types of floral organs can potentially develop in any of the four floral whorls. One of the few floral architectures that may not be easily obtainable is a flower with a reverse organ order (i.e. carpels, stamens, petals, sepals). This may be due to a combination of the antagonistic nature of the A and C activities, and that the A class genes are also involved in earlier events in floral development, namely the specification of the flower meristem.

Flower Patterning Genes

While the ABC model successfully explains the specification of floral organ identity, several key questions remain. First, what is responsible for the initial expression of floral meristem identity genes? Are these genes activated by the same factors that mediate the transition from vegetative to reproductive development? Second, how are the precise boundaries of the ABC gene expression patterns initiated and maintained? In the case of the A–C boundary, the mutual antagonism between the two activities helps to establish and maintain this boundary. Since A class activity is already present due to the earlier role played by the A class genes in flower meristem identity, the question of the A–C boundary is reduced to how C class activity is activated only in the third and fourth whorls. Although the details are not yet clear it seems that a combination of the meristem identity gene *LFY* and another factor localized to the third and fourth whorls are responsible for the initial activation of the C class gene, *AG*. In addition, it appears the meristem identity genes, particularly *LFY*, also play a role in the initial activation of the B class genes. The restriction of B class expression to the second and third whorls is in part mediated by the *SUPERMAN* (*SUP*) gene, mutations in which result in B class activity expanding into cells that would normally comprise the fourth whorl. The phenotypic consequence of *sup* mutations is that additional stamens develop, at the expense of the carpels. And third, how does positional information encoded by the floral homeotic genes get translated into differentiation of different floral organs? Presently, little is known about the targets of floral homeotic gene regulation.

Summary

Flowers consist primarily of four basic organ types whose relative positions are universally conserved within the angiosperms. The ABC model, based on molecular genetic experiments, explains how a small number of regulatory genes, acting alone and in combination, can specify floral organ identity. Since mutations and genes similar to those described here have been found in many species of angiosperms, the ABC model of floral organ identity specification is likely to hold for all species of flowering plants. Similarly, genes that specify flower meristem identity have been found in evolutionarily divergent plants, suggesting that this process is also governed by a common genetic programme in the angiosperms. While the ABC model successfully explains the specification of organ identity, it does not address how the numbers, positions, and shapes of the floral organs are established. Since these are features that distinguish flowers of different species, the mechanisms establishing these parameters are likely due to

genetic pathways that are downstream, or independent, of the universal genetic programme that specifies floral organ identity.

Further Reading

Bowman JL (1997) Evolutionary conservation of angiosperm flower development at the molecular and genetic levels. *Journal of Biosciences* **22**: 515–527.

Coen ES and Meyerowitz EM (1991) The war of the whorls: genetic interactions controlling flower development. *Nature* **353**: 31–37.

Riechmann JL and Meyerowitz EM (1997) MADS domain proteins in plant development. *Biological Chemistry* **378**: 1079–1101.

Meyerowitz EM (1994) The genetics of flower development. *Scientific American* November.

Meyerowitz EM, Smyth DR and Bowman JL (1989) Abnormal flowers and pattern formation in floral development. *Development* **106**: 209–217.

Schwarz-Sommer Z, Huijser P, Nacken W, Saedler H and Sommer H (1990) Genetic control of flower development: homeotic genes of *Antirrhinum majus*. *Science* **250**: 931–936.

Sessions A, Yanofsky MF and Weigel D (1998) Patterning the floral meristem. *Seminars in Cell and Developmental Biology* **9**: 221–226.

Weigel D (1995) The genetics of flower development: From floral induction to ovule morphogenesis. *Annual Review of Genetics* **29**: 19–39.

Weigel D and Meyerowitz EM (1994) The ABCs of floral homeotic genes. *Cell* **78**: 203–209.

Yanofsky MF (1995) Floral meristems to floral organs: Genes controlling early events in *Arabidopsis* flower development. *Annual Review of Plant Physiology and Plant Molecular Biology* **46**: 167–188.